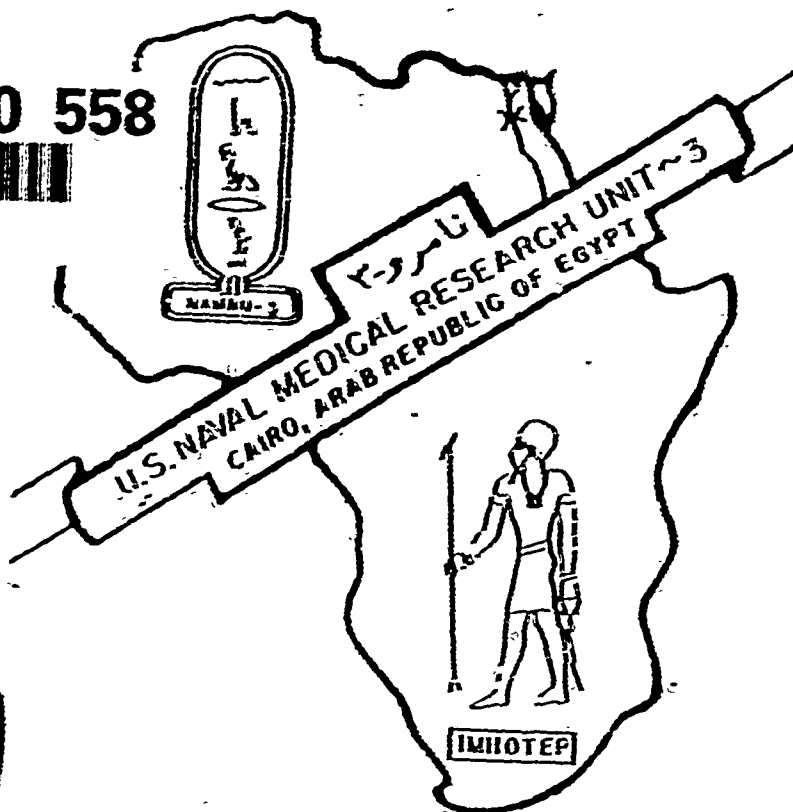


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DIARRHEA IN CHILDREN IN ASWAN, EGYPT

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Microbiologic and Clinical Study of Acute Diarrhea in Children in Aswan, Egypt

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Children with diarrhea presenting to a Government Rehydration Center in Aswan, Egypt, were investigated to determine the etiology and clinical presentation of acute childhood diarrhea in southern Egypt. Among 126 outpatients and 25 inpatients with diarrhea (mean age 18 months), enterotoxigenic *Escherichia coli* (ETEC) (17% of cases), *Cryptosporidium* (9%), *Salmonella* spp. (7%), *Campylobacter jejuni/coli* (7%), and *Shigella* spp. (5%) were the most common enteropathogens identified during the high incidence season of July. Enteropathogens were isolated as often from inpatients as outpatients, except for *Salmonella* spp. and *Cryptosporidium*, which were recovered more often from inpatients. *Salmonella*-infected children, in particular, were more ill, feverish, and dehydrated on presentation than other children, resulting in more frequent hospitalization. Except for *Salmonella*-infected children, children with acute diarrhea usually presented without severe dehydration, which may have been due to frequent initiation of oral rehydration therapy (ORT) by mothers trained by local health care providers. A potential environmental source of ETEC was identified in clay water storage containers commonly used in this area.

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INTRODUCTION

Diarrheal disease is a major public health problem in most developing countries, especially in children (1, 2). In Egypt, enterotoxigenic *Escherichia coli* (ETEC), *Salmonella* spp., *Shigella* spp., and rotavirus have been found to be major causes of diarrhea (3, 4). In an effort to control diarrheal disease in children, the Egyptian Government has instituted an extensive oral rehydration program involving public education and training by local health care providers, television advertisements, and newspaper stories. The purpose of the present study was to investigate the etiology and clinical presentation of acute diarrhea in children in southern Egypt.

MATERIALS AND METHODS

During a 3 week period in July 1986, children with diarrhea presenting to the Government Rehydration Center in the Aswan General Hospital were investigated. The center is a primary care facility for the city of Aswan and sees few patients referred from other clinics. Most patients are from a low socioeconomic background, and in this population, children are usually breast-fed until approximately 1 year of age. July was chosen for the study because the hot summer months are considered the high incidence season for diarrhea in this area with a dry desert climate.

Children were eligible for inclusion in the study if 5 years of age or less and if presenting with the complaint of at least 4 unformed stools during the previous 24 h. Children who had received antibiotic therapy were not excluded from the study. Children requiring hospitalization from the rehydration center were considered inpatients; all other children not requiring hospitalization were classified as outpatients. Children ≤ 5 years without diarrhea or other gastrointestinal complaints presenting for

medical care at an outpatient pediatric clinic in the same hospital were eligible for inclusion in the study as controls.

After informed consent was obtained from a parent, a stool sample was collected and an epidemiologic questionnaire was completed on each patient. The questionnaire was administered to the parent, usually the mother, in Arabic by an Egyptian pediatrician who also evaluated the patient clinically. Stool cultures were transported to the microbiology laboratory and cultured within 1 h of collection.

Patient and control stool specimens were cultured directly onto MacConkey's (MAC), Salmonella-Shigella (SS), Hecktoen (HK), thiosulfate-citrate-bile salts-sucrose (TCBS) agars, and into selenite F enrichment broth. Selenite broth was subcultured onto MAC, HK and SS agars after overnight incubation (35°C). All plates were examined for *Salmonella* spp., *Shigella* spp., *Aeromonas hydrophila*, *Plesiomonas shigelloides*, and *Vibrio* spp., using standard microbiologic methods, the API 20E system (Analytab Products, Plainview, NY, USA), and commercial antisera when indicated (Difco, Detroit, MI, USA) (5). For the detection of *Campylobacter jejuni/coli*, stools were cultured on Blaser's selective medium, which was incubated at 42°C for 48 h under microaerophilic conditions (6). All bacterial pathogens were evaluated for their antimicrobial susceptibility patterns by the Bauer-Kirby method (7).

For the detection of ETEC, 5 lactose positive colonies with typical *E. coli* morphology were selected from MAC plates and maintained on individual nutrient agar slabs. The presence of heat-labile enterotoxin (LT) was determined by the Y-1 adrenal cell assay (8). Heat-stable enterotoxin (ST) was determined by the suckling mice assay (9). ETEC were identified by the production of either LT or ST. For the detection of enteroinvasive *E. coli* (EIEC), the standard Sereny test was employed, but to diminish the number of guinea pigs required, all *E. coli* strains were screened for lysine decarboxylase activity and motility and only those strains which were negative for both were evaluated (10). *Giardia lamblia* and *Entamoeba histolytica* were screened for by the merthiolate-iodine-formaldehyde-concentration (MIFC) method (11). Oocysts of *Cryptosporidium* were identified by microscopic examination of stool smears stained by a modified acid-fast method (5). Rotavirus was detected by enzyme immunoassay (Rotazyme, Abbott Laboratories, North Chicago, Ill., USA).

Environmental samples of clay pots, "Zeirs," used to store water in Aswan were made by dipping a sterile cup into the top of each Zeir in a manner similar to the usual method of obtaining drinking water. 100 ml of water obtained from each Zeir was filtered through a 0.45 micron filter, which was then cultured on M-Endo agar. Typical coliform growth was subcultured to MAC agar for identifica-

Table I. Comparison of enteric pathogens isolated from outpatients and inpatients

Enteropathogen	Outpatients			Inpatients			Total positive	
	No. tested	Positive		No. tested	Positive		No.	%
		No.	%		No.	%		
Shigella spp.	126	8	6.3	25	0		8	5.3
Salmonella spp. ^a	126	2	1.6	25	9	36.0	11	7.3
Campylobacter	126	8	6.3	25	2	8.0	10	6.6
ETEC ^b								
Both ST and LT positive	114	2	1.8	21	0		2	1.5
LT positive alone	114	5	4.4	21	2	9.5	7	5.2
ST positive alone	114	14	12.3	21	0		14	10.4
EIEC	21	0		6	0		0	
A. hydrophila	126	1	0.8	25	0		1	0.7
Rotavirus ^c	123	26	21.1	25	1	4.0	27	18.2
G. lamblia ^d	123	35	28.5	25	7	28.0	42	28.4
E. histolytica	123	1	0.8	25	0		1	0.7
Cryptosporidium ^e	126	6	4.8	25	7	28.0	13	8.6

^a $p < 0.001$, outpatients vs. inpatients, Fisher's exact test.

^b *E. coli* could not be isolated from all stool specimens.

^c $p < 0.05$, outpatients vs. inpatients, Fisher's exact test.

^d Insufficient stool was available for complete testing for rotavirus, *Giardia*, and *E. histolytica*.

^e $p < 0.01$, outpatients vs. inpatients, chi-square test.

tion and selection of *E. coli*. *E. coli* colonies were processed for heat-labile and heat-stable toxins using the same techniques described for stool analysis.

Statistical analyses were done using the chi-square test with Yates' correction or Fisher's exact test to test for differences between proportions. Comparisons of mean values were done using the Student's *t*-test or Wilcoxon rank sum test. Probabilities of <0.05 were considered statistically significant.

RESULTS

There were 126 outpatients and 25 inpatients entered into the study (mean age 17.7 months; range, 1-60 months). 64% of the patients were male and 36% were female. Approximately one-fourth of the parents with children eligible for the study were willing to give consent and wait long enough for their child to provide stool specimen. Children had complained of diarrhea for an average of 3.5 days prior to being seen and had an average of 5.7 stools during the previous 24 h. Antibiotics had been received by 17.2% of patients with diarrhea.

ETEC was the most commonly isolated bacterial pathogen (Table I). LT and/or ST producing strains were found in 17% of stools from children with diarrhea. Other common bacterial pathogens included *Salmonella* spp. (1 *S. typhi*, 10 *S. typhimurium*), found in 7% of stools; *C. jejuni/coli*, also found in 7% of stool specimens; and *Shigella* spp. (3 *S. dysenteriae*, 4 *S. flexneri*, 1 *S. boydii*), found in 5% of stools. *A. hydrophila* was isolated in just 1 patient with diarrhea, and EIEC was not isolated from any study subject.

Bacterial enteropathogens were identified in 31.8% of 151 diarrheal stools. Mixed infections were found in 5 patients. *Campylobacter* plus *Salmonella* and *Salmonella* plus ETEC was isolated in 2 inpatients. In 3 outpatients, *Campylobacter* plus ETEC was

Table II. Clinical characteristics for the major enteropathogens isolated from 151 patients with diarrhea

ORT=Oral rehydration therapy

Characteristic	Enteropathogen						
	Salmonella (n=11)	Shigella (n=8)	Campylobacter (n=10)	ETEC (n=23)	Cryptosporidium (n=13)	Giardia (n=42)	Rotavirus (n=27)
Age in months (mean)	23.3	31.3	14.0	13.5	10.6	20.4	14.8
Duration of symptoms in days (mean)	5.0	2.3	3.5	4.0	4.1	3.5	2.6
No. stools/24 h (mean)	5.4	6.3	5.6	5.2	7.2	5.2	5.9
Symptoms (% with complaint)							
Mucus in stools	63.6	37.5	60.0	17.4	30.7	38.1	25.9
Blood in stools	27.3	25.0	20.0	8.7	15.4	7.1	7.4
Abdominal pain	54.6	100.0	100.0	78.3	53.9	73.8	81.5
Fever	90.9	87.5	80.0	56.5	84.6	57.1	63.0
Vomiting	54.6	62.5	50.0	43.5	69.2	50.0	63.0
Severe dehydration (>10%)	90.9	0	10.0	8.7	7.7	2.4	0
Therapy prior to admission							
Antibiotics (% treated)	45.5	37.5	30.0	8.7	46.2	11.9	14.8
ORT (% treated)	63.6	25.0	30.0	21.7	46.2	16.7	11.1

isolated twice, and *Campylobacter* plus *Shigella* was found once. *Campylobacter* was thus involved in 4/5 mixed bacterial infections.

Bacterial pathogens were isolated as often from inpatients as outpatients, except for *Salmonella* spp., which was recovered significantly more often from inpatients than outpatients (36.0% vs. 1.6%, $P < 0.01$) (Table I). *Salmonella*-infected children were more feverish, clinically ill, and dehydrated on presentation than other children, which resulted in more frequent hospitalization. More than 90% of *salmonella*-infected children were found to be severely dehydrated ($>10\%$), a rare finding in other children presenting with diarrhea (Table II). Also, complaints of blood and mucus in stools were more often associated with *salmonella* infection compared to other enteropathogens. *Salmonella*- and *shigella*-infected children tended to be older (mean age 26.6 months) than other children with acute diarrhea (mean age 16.5 months).

Nearly all *salmonella* strains were resistant to antibiotics commonly used to treat diarrhea in this area, including tetracycline, trimethoprim-sulfamethoxazole, and ampicillin. In addition to multiple resistance found in *Salmonella* spp., significant resistance was observed in other bacterial isolates, including one *campylobacter* isolate which was resistant to erythromycin (Table III).

Oocysts of *Cryptosporidium* were found in the stools of 6 outpatients and 7 inpatients (Table I). Patients infected with *Cryptosporidium* tended to be slightly more ill and to have had a larger number of stools than other patients, which was reflected in a higher percentage of inpatients than outpatients with cryptosporidiosis (Table II). *Cryptosporidium* tended to be associated with the concurrent isolation of *Campylobacter*: in 2 outpatients and 2 inpatients *Cryptosporidium* was simultaneously identified with *Campylobacter*. Whether the family of the patient raised animals or kept animals at their homes was not associated with *Cryptosporidium*, *Campylobacter*, or any other enteropathogens isolated in this study.

All bacterial pathogens were isolated more frequently from patients than from controls. 53 bacterial pathogens were isolated from 151 diarrheal stools, compared to 2 pathogens (1 *S. typhimurium* and 1 *Campylobacter*) isolated from 28 control stools. Also, *Cryptosporidium* was not identified in any controls, and trophozoites of *E. histolytica* were found in only 1 outpatient with diarrhea. In contrast, rotavirus and *G. lamblia* were identified as frequently in stools from patients with diarrhea as from controls. Rotavirus was found in 18.2% of 148 diarrheal stools and in 14.8% of 27 control stools, and *Giardia* was found in 28.4% of 148 diarrheal stools and in 32.1% of 28 control stools. Direct comparison between patients and controls was not possible, however, because control patients with-

Table III. Resistance patterns of the most commonly isolated enteric pathogens

ND = not done

Isolate	No. of resistant strains/no. examined (%)			
	Ampicillin	Tetracycline	Chloramphenicol	Trimethoprim/sulfa
<i>Salmonella</i> spp. ^a	10/11 (90.9)	10/11 (90.9)	10/11 (90.9)	10/11 (90.9)
<i>Shigella</i> spp.	3/8 (37.5)	3/8 (37.5)	3/8 (37.5)	1/8 (12.5)
ETEC	13/23 (56.5)	5/23 (21.7)	1/23 (4.3)	7/23 (30.4)
<i>Campylobacter</i>	4/10 (40.0)	2/10 (20.0)	ND	10/10 (100.0)

^a All 10 resistant *Salmonella* were *S. typhimurium*

out diarrhea were significantly older (mean age 42 months) than patients with diarrhea (mean age 18 months).

Analysis of epidemiologic data indicated that of patients with stools positive for ETEC, a higher percentage (78%) lacked an indoor home water faucet compared to patients with other causes of diarrhea (42%) and controls (21%; $p < 0.01$, both comparisons). Because of the possibility that a common form of water storage in clay pots (Zeirs) could be a source of diarrheal pathogens, a random survey of 18 Zeirs located in areas where patients lived was made 1 year after the initial study. Numerous coliforms were identified in 10/18 Zeirs, and in 2, ST producing *E. coli* were found.

There were no deaths during the study period, and none of the outpatients, who were all treated with oral rehydration therapy (ORT) as recommended by the World Health Organization, later required hospitalization for diarrhea or dehydration. This may have been due in part to the fact that most patients with acute diarrhea presented without severe dehydration because mothers frequently instituted ORT at home (25% of patients). Only in salmonella-infected children did ORT instituted at home not appear adequate to prevent dehydration (Table II).

DISCUSSION

Similar enteropathogens were found in this population with diarrhea in southern Egypt as have been found in northern Egypt (3, 4). ETEC was the most common bacterial pathogen isolated, with *Salmonella* spp., *Campylobacter*, and *Shigella* less commonly found. *Cryptosporidium*, an enteric pathogen which has only recently been looked for in Egypt, was also found to be a potential significant cause of diarrhea in children (12, 13).

Although the control group was not strictly comparable to the diarrheal group, *Giardia* was identified as frequently in controls as in cases of acute diarrhea. *G. lamblia* has been found in a high percentage of asymptomatic controls in other studies conducted in Egypt, which suggests that infection with *Giardia* may not always cause acute diarrhea in this population (3). The fact that rotavirus was also found equally as often in cases as in older controls was contrary to the findings of previous studies conducted in Egypt, but similar to reports from other areas (3, 4, 14, 15). However, only 4 patients in the control group were positive for rotavirus, making comparisons difficult.

Finding *Salmonella* spp. and *Cryptosporidium* more often in patients requiring hospitalization suggests that these 2 organisms may be more pathogenic than other causes of diarrhea. *Salmonella* has previously been found to be a cause of fatal and potentially fatal diarrhea in infants in Egypt (4). The role of *Campylobacter* as a cause of acute diarrhea, in contrast, was less clear because it was isolated as a single pathogen in only 5/11 diarrheal stools positive for *Campylobacter*. The frequent identification of *Cryptosporidium* and *Campylobacter* together may indicate similar sources and methods of transmission, possibly from infected animals (16).

Finding more patients in this study without a home water tap infected with ETEC prompted a search for a common source of infection. Water is frequently stored for household and community use in Aswan in large porous clay pots, Zeirs, covered by loose-fitting wooden lids which allow for easy contamination. Usually located in front of houses and along roads, Zeirs provide public drinking water kept cold by evaporation of water through the sides of the porous pots. For people who do not have water piped into their home and have to carry water from a community water tap, Zeirs may be a primary source of water for household use. The isolation of ETEC in water storage jars, therefore, suggests one possible source of diarrheal infection. Although causality could not be demonstrated in this study, examples of waterborne outbreaks of ETEC have been

reported, lending support to this possibility (17, 18). Alternatively, the lack of a home water tap may indicate a lower socioeconomic level and other risk factors for the acquisition of ETEC (14).

The most gratifying finding of the study was the high level of understanding and practical use of oral rehydration therapy by mothers of young children in Aswan. Mothers of patients were not just aware of the benefits of ORT but were knowledgeable enough to request the government supplied rehydration packets and use them effectively. Mothers were, in fact, so knowledgeable about the benefits of ORT that many were unwilling to wait for their child to produce a stool specimen because they did not see a need. This demonstrates the progress that can be made in implementing public health measures in developing countries when a concerted effort is made to educate the public (19).

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